PILOT: MONITORING WEST NILE VIRUS IN WISCONSIN'S RUFFED GROUSE POPULATION

3 August 2018

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Objectives

- 1. Assess the feasibility of utilizing hunter-harvested ruffed grouse to obtain biological samples from harvested birds for disease screening and collecting relevant metadata.
- 2. Determine prevalence of exposure to West Nile virus (WNV) in ruffed grouse populations and if there is significant change by year in Wisconsin.
- 3. Evaluate if samples can be collected in sufficient numbers to assess prevalence across different regions of the state.
- 4. Examine submitted samples for evidence of clinical disease associated with WNV infection.

Background

Data from roadside surveys indicated spring 2017 ruffed grouse (*Bonasa umbellus*) drumming activity increased 17% statewide in Wisconsin compared to 2016 results (Dhuey 2017). Brood observations were also up approximately 16.5% from 2016, albeit still 44% below the long-term average. Despite encouraging surveys that suggested a possibility for increased harvest opportunities for fall 2017, many hunters reported fewer flushes while afield. Similar reports have been conveyed from hunters and management agency staff in Minnesota and Michigan.

Despite brood-rearing conditions being less than ideal in Wisconsin given the substantial summer rains in both 2016 and 2017, additional speculation about potential disease exposure has been recently circulating amongst media outlets. More specifically, West Nile virus (WNV), a mosquito-borne disease that causes encephalitis in the brain and myocarditis in infected individuals, has been suggested to be an additive-stressor to mortality and a possible reason for recent reductions in ruffed grouse numbers in Pennsylvania (Stauffer et al. 2017). West Nile virus has been reported in many bird species since first detected in the United States in 1999, including ruffed grouse (Centers for Disease Control and Prevention, 2017). Some infected birds readily die from infection, including Corvids such as American crows (*Corvus brachyrhynchos*)

and blue jays (*Cyanocitta cristata*), but most bird species are able to survive with WNV infection.

Experimental infection of ruffed grouse and greater-sage grouse (*Centrocercus urophanianus*) with WNV in a controlled laboratory setting has previously demonstrated the susceptibility of these species to the disease (Clark et al. 2006, Nemeth et al. 2017); yet it remains highly speculative if WNV is having a population-level impact on wild ruffed grouse. Researchers in Pennsylvania began statewide serosurveys for WNV from hunter-harvested ruffed grouse and found apparent prevalence rates of 14% (n = 202) and 22% (n = 217) in 2016 and 2017, respectively (J. Brown and L. Williams, unpublished data). Michigan Department of Natural Resources has also confirmed 11 cases of WNV-positive ruffed grouse in 2017 from hunter-harvested birds (T. Cooley, unpublished data); 1 WNV-positive grouse was confirmed in Minnesota in the early 2000s (C. Roy, personal communication), and is yet to be confirmed in Wisconsin.

Heightened concern surrounding potential impacts of WNV on wild ruffed grouse populations in Wisconsin occurred after a suboptimal 2017 hunting season. Roadside surveys conducted in spring 2017 indicated ruffed grouse drumming activity increased 17% statewide (Dhuey 2017), and hunters expected good experiences afield come the fall hunting season. However, spring drumming surveys provide an index to breeding population size and occur before annual production, and thus does not necessarily forecast juvenile recruitment into the fall population. Past research has indicated fall harvest of ruffed grouse is comprised mostly of juveniles (Dorney 1963), therefore poor annual production can adversely impact hunter experiences.

We propose a proactive-approach to assess prevalence of WNV in Wisconsin's ruffed grouse population by requesting hunters to provide samples from their harvested bird(s) for examination of disease exposure. This monitoring project would provide the first information of WNV prevalence in Wisconsin's ruffed grouse and provide valuable insight in the geographical distribution of the disease. Information collected can help determine exposure of grouse to WNV and if an intensive survival study should be initiated with WNV being one of many incorporated variables. Notably, this monitoring study will also be part of a multi-state collaborative effort involving wildlife biologists and wildlife disease staff from other natural resource agencies in the Great Lakes Region, as well as the Ruffed Grouse Society and Wisconsin Conservation Congress. This joint effort will provide a more comprehensive view of the role of WNV in the region than any individual state could execute alone, and demonstrate the interest of regional biologists in responding to hunter concerns.

Methods

Timeline and sample size:

Because annual outbreaks of WNV can be highly variable, we propose conducting this pilot monitoring project for a period of at least 3 years, 2018–2020. Our goal would be to collect 400 samples annually, with priority being to collect samples from the primary grouse range in northern and central regions of Wisconsin (Fig. 1). Samples from the southeast and southwest

regions will also be accepted. Sampling period for 2018 would be 15 September through 31 October, with emphasis on birds harvested as close to the late-summer mosquito season as possible.

Hunter involvement:

We will be working with Ruffed Grouse Society and the Wisconsin Conservation Congress to identify hunters willing to provide samples. We will also provide an opportunity for the public to request kits through their local Wildlife Management staff. We plan to target avid grouse hunters that spend considerable time pursuing grouse each fall, as well as individuals that hunt several locations within the state.

Sample collection:

Hunters will be provided with a sampling kit, which will contain:

- Sampling instructions
- 1 Nobuto strip for blood collection
- 1 plastic jar for whole heart collection
- 2 cold packs
- Insulated bubble envelope
- Disposable nitrile gloves
- Datasheet
- Prepaid/preaddressed shipping envelope

Hunters will be asked to record date and time of harvest, location of harvest (County; Township, Range, Section; GPS coordinates; or distance and direction from nearest town), age-class (juvenile or adult), sex, general body condition (e.g., thin, normal, fat), note any abnormal behavior, and provide their contact information (optional). Samples should be kept cool or refrigerated and mailed within 24 hours of collection to the WDNR Science Operations Center in Madison. After the grouse hunting season closes and all samples are received by WDNR staff, they will be submitted to the Southeastern Cooperative Wildlife Disease Study (SCWDS) at the University of Georgia, in Athens, GA for diagnostic testing. Heart samples will be tested for presence of WNV by the polymerase chain reaction (PCR) and if the virus is present, histological examination of the tissue will be performed. Nobuto strips will be reconstituted and exposure to WNV will be reported with a serologic titer value. Final results may not be available until after diagnostic testing by SCWDS concludes, and hunters can be emailed test results for their individual bird(s) if desired. Collection methodology will be similar for Minnesota and Michigan samples.

Analysis:

Prevalence of exposure to WNV of birds collected will be calculated using the number of positive detections from serum collected with Nobuto strips relative to the total number of blood samples collected. Further regional evaluation and age class prevalence estimates will be

assessed separately and will depend on samples submitted. Paired heart samples will be used to estimate histologic lesion scores as compared to antibody response through Nobuto strip analysis and WNV genetic presence through PCR.

Management Implications

Spring drumming-count surveys are used to monitor breeding ruffed grouse activity and provide an index to population trends in Wisconsin. Annual survey results have been also used to forecast fall hunting expectations. Monitoring prevalence of WNV in harvest ruffed grouse will provide valuable information on disease exposure and geographical distribution. If exposure to WNV is found to coincide with reductions in Wisconsin's ruffed grouse population, informing hunters may temper fall hunting expectations. Additionally, monitoring results could help to initiate an intensive survival and productivity study to investigate cause of mortality, with WNV being one of many incorporated variables.

Literature Cited

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Estimated budget:

Sampling Kits/Supplies	Unit	Price/unit	1 Year		3 Years	
			Quantity	Total	Quantity	Total
Nobuto strips	100/box	\$32.08	4	\$128.32	12	\$384.96
Primary sample bags – Nobuto strips	1,000/carton	\$30.00	1	\$30.00	2	\$60.00
Primary sample containers – hearts	500/case	\$107.43	1	\$107.43	2	\$214.86
Secondary sample bags – hearts	1,000/carton	\$21.00	1	\$21.00	2	\$42.00
Ice packs (2/kit)	96/case	\$21.00	9	\$189.00	9	\$189.00
Insulated envelopes	100/case	\$40.00	4	\$160.00	12	\$480.00
Shipping envelope	100/case	\$32.00	4	\$128.00	12	\$384.00
Disposable gloves	100/box	\$3.82	8	\$30.56	16	\$61.12
Shipping box for kit supplies	Each	\$0.37	400	\$148.00	1,200	\$444.00
Shipping – kits to hunters	Each	\$5.50	400	\$2,200.00	1,200	\$6,600.00
Shipping – prepaid return labels	Each	\$5.00	400	\$2,000.00	1,200	\$6,000.00
Exempt animal specimen labels	1,000/roll	\$38.39	1	\$38.39	2	\$76.78
Avery labels for kit ID# (5/kit)	3,000/box	\$45.28	1	\$45.28	1	\$45.28
Datasheets/instructions						
Paper towels						
		Subtotal: Sampling kit costs		\$5,225.98		\$14,982.00

			1 Y	Tear	3 Years	
Diagnostic Fees/Testing Costs	Unit	Price/unit	Quantity	Total	Quantity	Total
Nobuto strips		\$15.00	400	\$6,000.00	1,200	\$18,000.00
Hearts – PCR test		\$20.50	400	\$8,200.00	1,200	\$24,600.00
Hearts – histopath		\$50.00	40	\$2,000.00	120	\$6,000.00
Formalin	2.5 gallons	\$78.63	2	\$157.26	6	\$471.78
Kapaks for shipping formalin/hearts	1,000/case	\$464.34	1	\$464.34	2	\$928.68
Shipping to SCWDS		\$86.00	1	\$86.00	3	\$258.00
		Subtotal: Testing costs TOTAL COSTS		\$16,907.60		\$50,258.46
				\$22,133.58		\$65,240.46

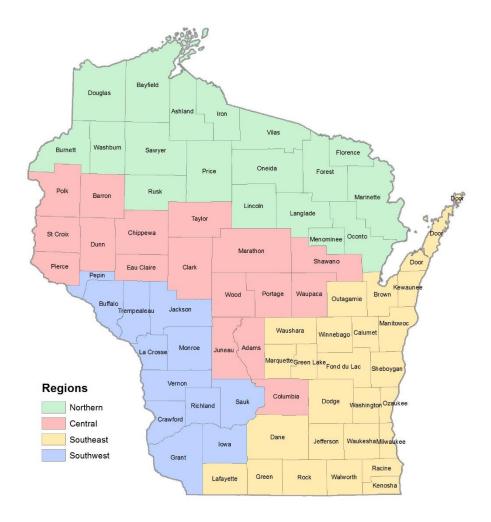


Figure 1. Wisconsin ruffed grouse drumming survey regions.